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CARBON DIOXIDE EVOLUTION OF FUNGICIDE-TREATED HIGH-MOISTURE CORN

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ABSTRACT. *Two corn hybrids, one resistant (FR35 × FR20) the other susceptible (DF20 × DF12) to storage fungi, were harvested and hand-shelled at 22% moisture, wet basis, and stored at this moisture in aerated l-kg bin units. Four Rovral® fungicide treatments plus an untreated control were tested using carbon dioxide evolution as the index of grain-deterioration rate. Equations of carbon dioxide weight versus time were fitted. The resistant corn hybrid manifested a lower deterioration rate than did the susceptible hybrid. Samples treated with fungicide showed a reduction in grain-deterioration rate compared with untreated samples.* **Keywords.** *Corn, Fungicide, Carbon dioxide, Iprodione.*

Carbon dioxide production was used by Bailey and Gurjar (1918) as a quantitative method for measuring the rate of respiration in wheat. They sealed wheat samples of known moisture content in jars and used barium hydroxide solution to absorb CO_2. The respiratory rate was expressed in terms of mg CO_2 respired per 100 g dry matter per 24 h.

Gross respiration of a grain mass under aerobic conditions has been modeled as the complete oxidation of a carbohydrate (Steel et al., 1969) and is represented by equation 1 (oxidation of glucose):

$$C_6H_{12}O_6 + 6O_2 \text{ ----> } 6CO_2 + 6H_2O + 2{,}835 \text{ kJ} \quad (1)$$

According to equation 1, a 1.0% loss in grain dry matter (carbohydrate) is accompanied by the evolution of 14.7 g CO_2/kg of grain dry matter. The carbon dioxide produced here can be used as an index of the deterioration rate of stored corn (Saul and Lind, 1958; Saul and Steele, 1966; Steele, 1967; Fawole, 1969; Seitz et al., 1982; Fernandez et al., 1985; Friday et al., 1989). According to Saul and Steele (1966), corn can lose up to 0.5% of its original dry matter or 7.35 g CO_2/kg dry matter through deterioration before grain quality is reduced by one commercial grade because of damaged kernels.

Steele et al. (1969) studied factors affecting CO_2 evolution by both grain respiration and microorganism growth; these factors included grain moisture content, grain-storage temperature, and mechanical damage. Thompson's computer simulation model (Thompson, 1972) used equation 2 from Steele et al. (1969) to calculate weight of CO_2 produced at the "standard" conditions of 15.5° C (60° F), 25% moisture, and 30% mechanical damage:

$$y = 1.3\,[\exp(0.006t) - 1] + 0.015t \quad (2)$$

where y = g of CO_2 produced per kilogram of original dry matter, and t = time, h. Under "nonstandard" conditions, the time required to produce a given amount of CO_2 was predicted from equation 3:

$$T = T_r \times M_m \times M_t \times M_d \quad (3)$$

where

- T = estimated time (h) to produce a given amount of CO_2
- T_r = time (h) to produce that amount of CO_2 at standard conditions
- M_m = moisture multiplier
- M_t = temperature multiplier
- M_d = damage multiplier; see Thompson (1972) for multiplier definitions

Grain hybrid is another factor contributing to grain-deterioration rate. Tuite et al. (1967) and Cantone et al. (1983) conducted laboratory storage tests of different corn hybrids and found significant differences in resistance to storage fungi. Friday et al. (1989) studied the effect of corn hybrid on carbon dioxide production during the storage of high-moisture shelled corn. The CO_2 evolution tests conducted on different corn hybrids showed significant differences in storability. Carbon dioxide production for resistant hybrids FRB73×M017 and FR35×FR20 was significantly less than that for either susceptible hybrid, DF20×DF12 or Pioneer 3377.

Treating grain with preservative chemicals can reduce grain deterioration rate. Niles (1980), in a study of fungi in stored barley treated with systemic fungicides, applied six different fungicides and found that Benomyl was the most effective. White et al. (1988) used different fungicides to delay fungi development in shelled corn being dried with natural air in a bin. One part of this study involved

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comparison of an untreated bin with a bin treated with MERTECT 340-F mixed with different carriers. These researchers reported that one of the treatments (MERTECT 340-F with water carrier) was acceptable for emergency use on Illinois farms in the fall of 1986 and 1987.

At present, Rovral® fungicide, which contains the active ingredient Iprodione (3-(3,5-dichlorophenyl)-N-(l-methylethyl)-2-4-dioxo-1-imidazolidinecarboxamide) and which was developed by Rhone-Poulenc Ag Company, is being studied to determine if it is effective against fungi development in high-moisture corn. The ingredients of this fungicide were so formulated to be effective against the two major storage fungi: *Aspergillus* and *Penicillium* spp. (Rhone-Poulenc Ag Company, 1990).

McGee (1990) treated corn with soybean oil with and without Rovral® fungicide to determine effects on storage fungi invasion. The study showed that treatment with soybean oil alone can reduce growth of storage fungi in corn stored at moisture contents of 17 and 15%, but it is not as effective as treatment with oil plus Rovral® fungicide. Effects of Rovral® fungicide have not been studied by using the criterion of CO_2 production.

Objectives

The objectives of this study were to:

- Quantify effects on CO_2 production of various Rovral® fungicide treatments of two corn hybrids (one resistant, one susceptible to fungi) and interactions between fungicide treatments and hybrids.
- Define equations of CO_2 production versus time for susceptible and resistant corn hybrids tested with various Rovral® fungicide treatments.

Experimental Procedures

Hybrid Selection

The choice of hybrids for this research was based on the study conducted by Friday (1987). Two hybrids were selected; one had been shown to be resistant and the other to be susceptible to storage fungi. The selected resistant hybrid, FR35 × FR20, demonstrated the smallest dry-matter loss rate of all the hybrids studied by Friday, and the susceptible hybrid, DF20 × DF12, demonstrated the greatest dry-matter loss rate.

The two selected hybrids were planted in May 1990 at the Agronomy and Agricultural Engineering Research Center, 15 km west of Ames, Iowa. They were hand-harvested in October 1990 when they had reached about 22% moisture (wet basis).

Sample Preparation

After harvesting, kernels were removed from cobs by hand-shelling. The samples were then passed through a Kice miniaspirator to remove any fines or light material by using the air velocity level (16 m/s), recommended by Al-Yahya et al. (1991). All damaged kernels such as large broken kernels and those damaged by insects or infected by field fungi were also removed. The remaining kernels were assumed to have 3% mechanical damage (Saul and Steele, 1966).

The samples were kept at 4° C for about three days and were then transferred into −10° C storage and kept at this condition until testing. According to Fernandez et al. (1985), corn stored at −10° C responds to storage fungi almost the same as freshly harvested corn. Samples were thawed at room temperature for 6 h prior to fungicide application.

Experiment 1 — Fungicide Application

Treatments are defined in table 1. Each treatment was applied to a 1300-g corn sample. Water was added so that the liquid application rate was 3.48 mL/kg (3 fl oz/bu). Water alone was applied to treatments 1 and 2.

Each 1300-g corn sample was spread in a 20 × 35-cm dish, and fungicide solution was sprayed on the corn with a l-mL syringe to ensure that each corn kernel was treated with fungicide. Each sample was then placed in a plastic bag and shaken for 3 min. A 150-g sample was removed from each treatment for moisture determination and other tests. The remaining grain was used for the CO_2 test.

Experiment 2 — Carrier Test Procedure

Treatment with fungicide involves not only the fungicide compound itself, but also the water, oil, or surfactant carrier. McGee (1990) found a fungicidal effect from soybean oil using biological tests. A three-treatment experiment was conducted on the susceptible hybrid to verify McGee's results using carbon dioxide evolution tests. Experimental procedures were the same as described for the first experiment. Treatment 1: Rovral + water + oil; Treatment 2: water + oil; Treatment 3: untreated control. A randomized complete block design was used, with two replications; treatments were randomly allocated to the corn samples.

Carbon Dioxide Production

The carbon dioxide absorption technique was used to measure the carbon dioxide production of the corn samples. The experimental setup used here was similar to those of Friday et al. (1989), Fernandez et al. (1985), and Steele et al. (1969). The apparatus consisted of 10 complete carbon dioxide absorption systems in a room controlled at 26° C (79° F). Aeration air came from a building compressed air line. The system (fig. l) includes the following components:

Carbon Dioxide Removal. Carbon dioxide in the incoming air was removed by bubbling the airstream through a 25% potassium hydroxide solution in a Drechel gas-washing bottle. Complete removal of CO_2 after passing through the potassium-hydroxide solution was

Table 1. Treatment definitions

Treatment	Hybrid*	Fungicide Treatment†	Weight Ratio
1	FR35×FR20	water (control)	
2	DF20×DF12	water (control)	
3	FR35×FR20	Rovral:water	1:83
4	FR35×FR20	Rovral:water:soybean oil	1:79:4
5	FR35×FR20	Rovral:water:soybean oil (half dose)	1:81:2
6	FR35×FR20	Rovral:water:activator 90	1:83:0.25
7	DF20×DF12	Rovral:water	1:83
8	DF20×DF12	Rovral:water:soybean oil	1:79:4
9	DF20×DF12	Rovral:water:soybean oil (half dose)	1:81:2
10	DF20×DF12	Rovral:water:activator 90	1:83:0.25

* Hybrid (FR35×FR20) is resistant and hybrid (DF20×DF12) is susceptible to storage fungi.

† Rovral was applied at a rate of 20 ppm (weight of Rovral / wet weight of corn).

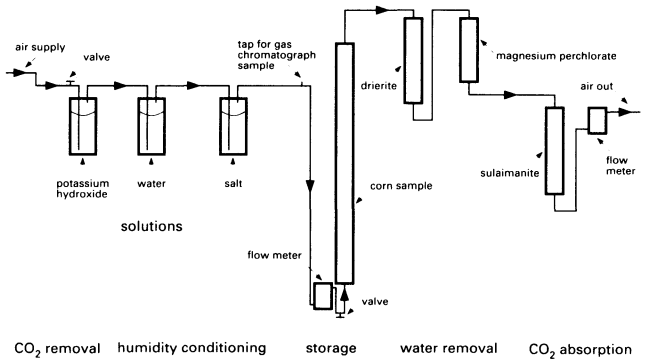

Figure 1–Carbon dioxide measurement system.

verified by gas chromatography on 50-mL samples. The potassium-hydroxide solution was changed every three days.

Humidification. The relative humidity of the airstream was controlled by bubbling the airstream through two 250-mL Drechel gas-washing bottles in series. The first bottle was filled with water and the second with a saturated potassium sulfate solution. This salt solution conditioned the air to about 97% relative humidity. This air maintained the corn at a moisture content of approximately 21.5% throughout the experiment.

Sample Storage and Aeration. The sample storage and aeration component consisted of a 122-cm (4-ft) long and 4.44-cm (1.75-in.) internal diameter plexiglas tube, which used 5 cm (2 in.) of fiberglass as a permeable floor. Air passing through the storage unit was controlled by both a manifold air-distribution unit and a needle valve and monitored by Matheson Model PM-1022 Acrylic Purge Flowmeters calibrated with a Gilmont No. 12 flowmeter. Airflow rates were set at 0.45 m^3/min-t (0.4 cfm/bu) of corn throughout storage. The system was checked for air leaks at intervals of approximately 6 to 8 h.

Water Absorption. H_2O and CO_2 are produced during grain and microorganism respiration, and are carried out by air passing through the storage unit. In this experiment, two drying agents were used to absorb water vapor. The first agent was a 1:1 mixture of 8-mesh drierite (anhydrous $CaSO_4$) and 8-mesh indicating drierite (97% $CaSO_4$ and 3% $CoCl_2$) which turns from light blue to pink as it absorbs water. It was placed in a plexiglas tube 46 cm (18 in.) long and 2.54 cm (1 in.) in diameter. The airstream was then passed through the second agent, $Mg[ClO_4]_2$, which was placed in a plexiglas tube 30.5 cm (12 in.) long and 2.54 cm (1 in.) in diameter. This second drying agent was used only to ascertain whether any water passed untrapped through the first agent. Because the second drying agent did not change color when it absorbed water, 5 cm (2 in.) of the first drying agent was placed at the bottom of the second drying agent as a further indicator of unabsorbed water.

Table 2. Equations of CO_2 production (g/kg dry matter) by susceptible corn hybrid DF20 × DF12 vs. time (h) after different treatments of fungicide

Treatment	Equation
Rov.+H_2O	–0.2997+0.02412*t–0.000051*t^2+0.00000013*t^3
Rov.+H_2O+Oil	–0.0723+0.020234*t–0.000039*t^2+0.00000010*t^3
Rov+H_2O+1/2 Oil	–0.0982+0.020941*t–0.000047*t^2+0.00000013*t^3
Rov+H_2O+Activ.	–0.2217+0.019847*t–0.000036*t^2+0.00000010*t^3
H_2O (Untreated)	0.35024+0.00654*t+0.000072*t^2+0.00000002*t^3

Carbon Dioxide Absorption. For this component, the ascarite used in previous studies for absorbing CO_2 was replaced by sulaimanite, a mixture of vermiculite and potassium hydroxide solution (Al-Yahya, 1991). CO_2 in the airstream was absorbed in sulaimanite in a plexiglas tube 46 cm (18 in.) long and 2.54 cm (1 in.) in diameter. Segments of drying agents drierite and magnesium perchlorate were placed below the sulaimanite in the column to absorb water liberated from the sulaimanite, according to the chemical equation:

$$2KOH + CO_2 \text{ ----} > K_2CO_3 + H_2O$$

The carbon dioxide absorption column contained 30.5 cm of sulaimanite (top layer), 5 cm of magnesium perchlorate (middle layer), and 10 cm of drierite (bottom layer). Readings of CO_2 weight were taken every 6 to 15 h throughout the tests. The sulaimanite columns were changed every three to four days, their color changing from dark to light gray upon absorption of CO_2. We assumed that all CO_2 was trapped by this system. This assumption was verified by gas chromatograph analysis of gas samples

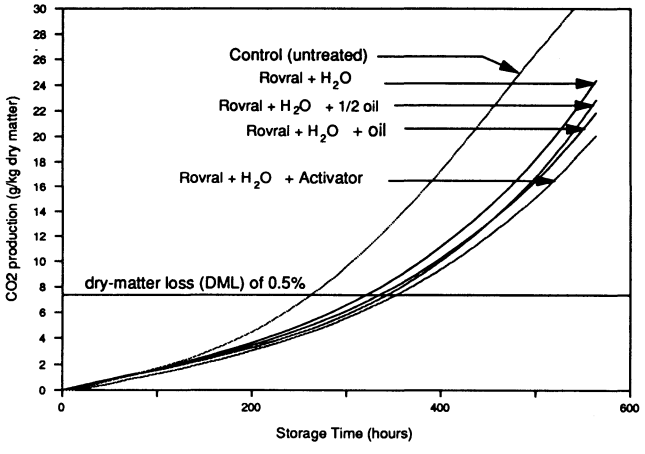

Figure 2–Carbon dioxide produced by susceptible corn hybrid DF20 × DF12 untreated and treated with different fungicides. (Curves are third-order polynomial equations from table 2.)

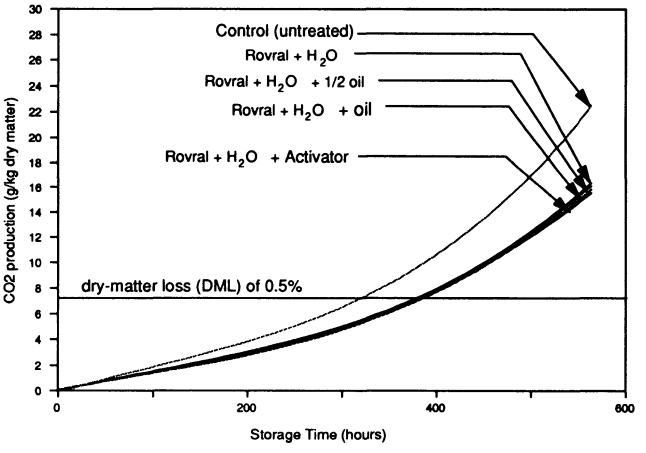

Figure 3–Carbon dioxide produced by resistant corn hybrid (FR35 × FR20) untreated and treated with different fungicides. (Curves are third-order polynomial equations from table 3.)

Table 3. Equations of CO_2 production (g/kg dry matter) vs. time (h) by resistant corn hybrid FR35 × FR20 after treatments of fungicide

Treatment	Equation
Rov.+H_2O	−0.14185+0.02060*t−0.000044*t^2+0.00000009*t^3
Rov.+H_2O+Oil	−0.065+0.0180200*t−0.000027*t^2+0.00000007*t^3
Rov+H_2O+1/2 Oil	−0.1099+0.018360*t−0.000035*t^2+0.00000008*t^3
Rov+H_2O+Activ.	0.01063+0.01571*t−0.000025*t^2+0.00000007*t^3
H_2O (Untreated)	−0.3289+0.027444*t−0.000062*t^2+0.00000013*t^3

drawn from the system at several times during testing. The CO_2 column was changed when weight had increased about 3 g.

After each test, the system was disassembled and cleaned in the following order: warm water, soap, warm water, and cold distilled water.

Statistical Analysis

A statistical analysis was done using the SAS statistical package on results of the main experiment, a randomized complete block design which included 10 treatment combinations consisting of two hybrids each with five Rovral® treatments. The study had two replications and was blocked in time. A two-way analysis of variance (ANOVA) was used for the data collected in the carbon dioxide tests. Orthogonal contrasts were used to make comparisons among treatment means (Snedecor and Cochran, 1989). Comparisons were made for resistant versus susceptible hybrids, fungicide treatments, control samples, and interactions between hybrids and fungicide treatments, for times required for the dry-matter loss to reach 0.5 and 1.0%.

Results and Discussion

Effect of Corn Hybrid on Carbon Dioxide Production

Equations of time versus carbon dioxide produced by fungicide-treated and control samples of susceptible hybrid are presented in figure 2 and table 2, and in figure 3 and table 3 for the resistant corn hybrid. Data from two replications were pooled. Mean storage times of untreated and treated susceptible and resistant corn hybrids at 0.5 and 1.0% DML are presented in table 4, along with least significant difference values. Statistical analysis results in the form of estimate contrasts among treatment means are presented in table 5 for times to reach 0.5 and 1.0% DML.

Table 4. Mean storage times to 0.5 and 1.0% DML for four fungicide treatments and control for two hybrids (resistant and susceptible)

	Hybrid			
	Susceptible		Resistant	
	Dry Matter Loss Level (%)			
	0.5	1.0	0.5	1.0
Treatment	Storage Time (h)			
Untreated (control)	262	393	328	491
Treated				
1-Rovral+H_2O	322	457	388	525
2-Rovral+H_2O+Oil	335	475	392	535
3-Rovral+H_2O+1/2 Oil	340	470	390	530
4-Rovral+H_2O+Activator	355	495	395	540
LSD*	19	18	19	18
Average of Rovral Treatments	338	475	391	533

* Least significant difference (p = 0.05).

Table 5. Estimated storage time values of comparisons among treatment means

	Dry Matter Loss Level (%)			
	0.5		1.0	
Contrast	(h)			
C1: Res.–Sus.	55.8*	(3.6)†	63.2*	(3.4)
C2: Rov.–(Rovral+carriers)	−12.8ns	(4.7)	−16.5*	(4.3)
C3: C1×C2	−8.5ns	(2.4)	−6.5ns	(2.2)
C4: Oil–1/2 oil	−1.5ns	(5.8)	−5.0ns	(5.3)
C5: C1×C4	3.5ns	(2.9)	0ns	(2.7)
C6: Oil–Activator	−10.8ns	(5.0)	−15‡	(4.6)
C7: C1×C6	6.8ns	(2.5)	7.5ns	(2.3)
C8: Rovral–control	69.6*	(4.4)	61.4*	(4.1)
C9: C1×C8	−6.3ns	(2.2)	−19.9*	(2.0)

ns Not significant.
* p<0.005.
† Values in parentheses are standard deviations of estimated values of contrasts.
‡ p<0.05.

From table 5, note that:

- The times to reach 0.5 and 1.0% DML for the resistant hybrid are significantly greater than the susceptible hybrid times.
- Times for Rovral®-treated samples are significantly greater than times for control samples at both 0.5 and 1.0% DML.
- Times for Rovral® + carrier samples are significantly greater than times for Rovral samples at 1.0% DML, but not at 0.5% DML.
- Times for activator-treated samples are significantly greater than times for oil-treated samples at 1.0% DML, but not at 0.5% DML.
- There is a significant interaction between hybrid and Rovral® treatment only at 1% DML.
- No other contrast comparisons are significant:
  - There is no significant interaction between hybrid and fungicide treatment.
  - There is no significant difference between oil and 1/2 oil.
  - There is no significant interaction between hybrid and oil treatment.
  - There is no significant interaction between hybrid and carrier (activator or oil).

Table 6. Storage time for untreated susceptible and resistant corn hybrids as predicted by Steele (1967), Friday (1987), and as observed in this study*

		Dry Matter Loss (%)			
		0.25	0.5	0.75	1.0
Study	Hybrid	(h)			
Steele					
	Susceptible	163	278	366	429
	Resistant	223	381	502	589
Friday					
	Susceptible	172	292	386	453
	Resistant	247	420	554	650
Current study					
	Susceptible	170	262	320	393
	Resistant	190	328	425	491

* Steele and Friday values have been adjusted to 3% damage, 26° C, 21.6% moisture, and multipliers of 0.91 and 1.25 have been applied to account for susceptibility and resistance, respectively.

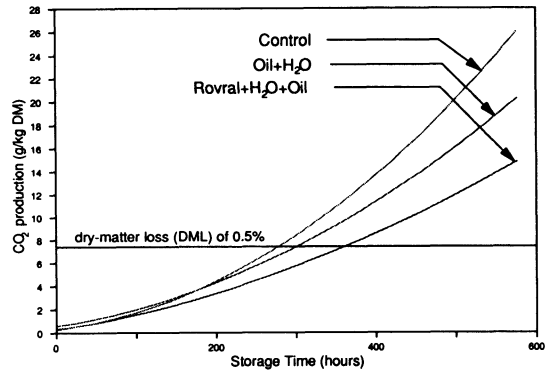

Figure 4–Carbon dioxide production of corn for carrier experiment. (Curves are third-order polynomial equations to fit the data.)

Steele and Friday Results Compared with Those of This Study

Storage time for untreated susceptible and resistant corn hybrids as predicted by Steele (1967) and Friday (1987) and as were observed in this study are compared in table 6. Values for the other studies were adjusted to the same conditions of the current study, i.e., 26° C (78.8° F) storage temperature, 21.6% corn moisture, 3% mechanical damage. The Steele data were also adjusted to account for hybrid susceptibility and resistance using multipliers of 0.91 and 1.25, respectively. These multipliers were calculated as the average ratio of storage times for susceptible and resistant corn in this study to Steele's times adjusted to our conditions. Times predicted using Friday's method reflect susceptibility and resistance multipliers that he had determined. Friday's predictions are consistently higher than times observed in the current study and times computed with Steele's equations. Except for susceptible corn at 0.25% DML, Steele's predicted times are also higher than those of this study, but are much closer to them than are Friday's predictions.

Effect of Carrier on CO_2 Production

Results of the carrier effects experiment are shown in figure 4 and tables 7, 8, and 9. Storage times to 0.5 and 1.0% DML are shown in table 8; statistical analysis results are shown in table 9. Times to 0.5 and 1.0% DML are significantly higher for oil-treated samples than for control samples, and times for oil + Rovral®-treated samples are significantly higher than for oil-treated samples.

Conclusions

The study led to the following conclusions:

- The resistant corn hybrid FR35 × FR20 lost 0.5 and 1.0% dry matter in significantly longer times than did susceptible hybrid DF20 × DF12.
- Corn samples treated with Rovral fungicide lost 0.5 and 1.0% dry matter in significantly longer times than those required for untreated corn samples. Treated sample times to 0.5 and 1.0% dry matter loss were 124 and 114% of untreated sample times, respectively.

Table 7. Equations of CO_2 production (g/kg dry matter) by susceptible hybrid DF20 × DF12 vs. time (h) after treatment with carrier, with or without Rovral®

Treatment	Equation
ROVRAL+OIL+WATER	0.263547+0.010384*t+0.000026*t^2
OIL+WATER	0.607069+0.010007*t+0.000042*t^2
CONTROL	0.347928+0.007528*t+0.000064*t^2

Table 8. Mean storage times for untreated, carrier treated, and Rovral® + carrier treated corn (susceptible hybrid)

	Dry Matter Loss (%)	
	0.5	1.0
	(h)	
Rovral+Oil+H_2O	367	566
Oil+H_2O	296	479
Control	267	422

Table 9. Estimated values [storage time (h) to 0.5 and 1.0% DML] of comparisons among treatment means in carrier experiment

	Dry Matter Loss (%)	
	0.5	1.0
Contrast	(h)	
C1: Control–(oil+H_2O)	–29* (3.6)	–57* (10)
C2: (Oil+H_2O)–(oil+Rovral+H_2O)	–71* (3.6)	–87* (10)

* $p<0.01$.
† Values in parentheses are standard deviations of estimated values of contrasts.

- Storage times observed in this study were similar but less than times predicted by previous studies of Steele and Friday for the same conditions.
- Fungicide carrier (oil) significantly extended the storage time to 0.5 and 1.0% dry matter loss, but not as much as treatment with oil + fungicide.

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